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*Article*

# Chemical composition and biological activity of essential oil from *Cymbopogon citratus* leaves on the quality of fresh orange juice during storage

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***Abstract***

*The present study aims to evaluate the effect of essential oil (EO) from Cymbopogon citratus leaves against the spoilage flora of fresh orange juice. Thus, the EO was extracted by hydrodistillation from fresh leaves of Cymbopogon citratus collected in southern Benin and its chemical composition was determined by gas chromatography, coupled to mass spectrometry (GC/MS). Orange samples were collected from large production areas of South and Central Benin and juices were extracted by mechanical pressing. After identification of spoilage flora of fresh orange juice, antimicrobial tests were carried out with the EO of Cymbopogon citratus to evaluate its antimicrobial activity on spoilage flora of fresh orange juice. Results indicate that the spoilage flora of fresh orange juice is mainly composed of fungi belonging to the genera of Cladosporium, Penicillium and Fusarium. Bacteria such as Enterobacter cloacae and Enterobacter aerogenes were also identified in some samples. The major compounds identified in the EO by GC/MS are Neral (33.0%) and geranial (41.3%) with a predominance of oxygenated monoterpenes (85.5%). Antimicrobial tests have revealed a high antibacterial activity of the EO, with minimum bactericidal concentrations (MBC) between 0.1 and 0.15 µL.mL-1. Antifungal tests revealed that fungi are also susceptible to this EO with minimum fungicidal concentration (MFC) between 0.15 and 0.25 µL.mL-1. Results obtained during the evaluation of the physicochemical characteristics of the orange juice stored by adding EO, indicated a significant decrease in the pH and vitamin C content. However, with EO concentration of 0.250 µL.mL-1, the pH of stored juice was 6.4 ± 0.1 after 15 days of preservation, with a best vitamin C content of 28.06 ± 0.03 mg / 100mL. The EO of Cymbopogon citratus, with high antimicrobial activity, could be used as an alternative in the preservation of fruit juices, replacing antimicrobials from chemical synthesis.*

# 1 Introduction

Generally, fruit juices are a good source of minerals, vitamins, trace elements and antioxidants. Fruits are also known to have nutritional and medicinal properties that can be attributed to their antioxidant effects and they can be used to fortify staple foods particularly for malnourished children (Barminas *et al.*, 1998). Orange juice has great nutritional importance because of its richness in nutrients including vitamins, minerals, dietary fiber, organic acids and bioactive substances (Tran and Farid, 2004). However, because of this wealth, the orange juice is a preferred substrate for fermentation germs. To fight against these microorganisms, two preservation methods are often used by fruit juice producers in Africa: pasteurization and use of chemical synthetic preservatives. Pasteurization is a heat treatment to destroy pathogenic microorganisms, but also can change the nutritional quality of the juice, because of the sensitivity of certain nutrients like vitamins to heat. It is also known that the flavor of orange juice is influenced by heat treatment (Bazemore *et al.*, 1999). Similarly, in the absence of heat treatment, antimicrobials from chemical synthesis also used in food preservation. However, application of high concentrations of these synthetic chemicals in a conservation of food, increases the risk of toxic residues in food. Due to the increasing sensitivity of consumers in this residual pollution and toxic effects of many synthetic fungicides, the importance of using natural alternatives becomes necessary (Hidalgo *et al.*, 1998). Similarly, the restriction imposed by the food industry and regulatory agencies on the use of certain synthetic food additives have led to renewed interest in the search for alternatives, such as natural antimicrobial compounds, especially those of vegetable origin (Moosavy and Basti 2008). Essential oils and derivative compounds have important activities which the antimicrobial activity is the most studied (Bankole *et al.*, 2004). *Cymbopogon citratus*, Stapf (Lemon grass) is a widely used herb in tropical countries. The essential oil of the plant is used in aromatherapy. Early research reported that this essential oil, in agar plate, was active on *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* (Melo *et al.*, 2001). Other studies reported that the oil is also active against food storage fungi (Mishra and Dubey, 1994). Thus, the present study aims to evaluate the antimicrobial properties of the essential oil of *Cymbopogon citratus* L. against the spoilage flora of fresh orange juice in Benin.

# 2 Material and Methods

## *2.1 Collection of plant leaves*

Plant materials used for essential oil (EO) extraction were fresh leaves from *Cymbopogon citratus* L. Plants were collected at Abomey-calavi (south Benin) and identified at the Benin national herbarium, where voucher specimens are deposited.



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## 2.2 Essential oil extraction

The EO tested was extracted by the hydro-distillation method using Clevenger-type apparatus. The oil recovered was dried over anhydrous sodium sulfate and stored at 4 °C until it was used (de Billerbeck *et al.*,2001).

## 2.3 Gas chromatography–mass spectrometry analysis

The EO were analyzed by gas chromatograph (Perkin Elmer Auto XL GC; Waltham, MA, USA) equipped with a flame ionisation detector, and the GC conditions were EQUITY-5 column (60 m x 0.32 mm x 0.25 µm); $H_2$ as the carrier gas; column head pressure 10 psi; oven temperature program isotherm 2 min at 70 °C, 3 °C/min gradient 250 °C, isotherm 10 min; injection temperature, 250 °C; detector temperature 280 °C. Gas chromatography–mass spectrometry (GC-MS) analysis was performed using a Perkin Elmer Turbomass GC-MS. The GC column was EQUITY-5 (60 m x 0.32 mm x 0.25 µm); fused silica capillary column. The GC conditions were injection temperature, 250 °C; column temperature, isothermal at 70 °C for 2 min, then programmed to 250 °C at 37 °C/min and held at this temperature for 10 min; ion source temperature, 250 °C. Helium was the carrier gas. The effluent of the GC column was introduced directly into the source of MS and spectra obtained in the EI mode with 70 eV ionisation energy. The sector mass analyzer was set to scan from 40 to 500 amu for 22 s. The identification of individual compounds is based on their retention times, retention indices relative to $C_5$ – $C_{18}$ n-alkanes, and matching spectral peaks available in the published data (Adams, 2007).

## 2.4 Collection of oranges and juice extraction

Samples of oranges were collected in the main markets of localities of Allada (south of Benin), Klouekanme (south of Benin) and Zakpota (center of Benin) which are the major sales depot of oranges in Benin. In each locality, four markets, were investigated and samples were purchased from five different points, and were mixed together to give a composite samples from each market which were used for the analysis. The juice was extracted by mechanical pressing after pulping and cleaning fruits. Juices were collected and kept at 4 °C until microbiological analyses.

## 2.5 Microbiological analysis

For microbiological analysis, 25 g of each sample and 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated at 30°C for 72 h. Desoxycholate was used for the total coliforms count and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the faecal coliforms count. In this case, plates were incubated at 44°C and the identification was made using Eosine Methylene Blue (EMB) medium. Tryptone sulfite neomycin agar was used for anaerobic sulfito-reducer (ASR) count, and tubes were incubated at 37 °C for 24 h.



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After incubation, the number of colonies was tracked, using a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, considering dilution factor. All media used for microbiological analysis were prepared as indicated by the manufacturer. After their isolation, bacteria were also controlled with API System (BioMérieux France).

## *2.6 Fungal isolation and identification*

The isolation of fungi from samples was performed using dilution plating method. Ten gram of each juice sample were added separately to 90 mL of sterile water containing, 0.1% peptone water. This was thoroughly mixed to obtain the $10^{-1}$ dilution. Further, 10fold serial dilutions up to $10^{-4}$ were made. 1 mL volume of each dilution was separately placed in Petri dishes, over which, 10 to 15 mL of potato dextrose agar amended with 60 µg/mL chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2 °C for 7 days. Fungal isolates from PDAC were sub-cultured on malt extract agar (MEA), and identification was carried out by using a taxonomic schemes primarily based on morphological characters, using the methods described by (Singh *et al.*, 1992).

## *2.7 Biological assays*

### 2.7.1 Minimum inhibitory concentration (MIC)-broth microdilution method

To determine the MIC, broth microdilution method proposed by Bajpai *et al.* (2008) were used. The microdilutions on 96 well plates were used with Mueller Hinton Broth (MHB) and 0.02 g/L phenol red. EO and MHB constitute the negative control. The positive one is bacteria strain plus MHB. The microplates were incubated at 37 ± 1 °C for 24 h, covered with a parafilm paper.

### 2.7.2 Minimum bactericidal concentration (MBC)

MBC were appreciated by method proposed by Oussou *et al.*, (2004). To determine the MBC, each microliter-plate well in which no color change occurred was used. The mixture of EO and the strain was isolated on sterile MHA poured in Petri dishes. These plates were incubated at 37 °C for 24 h. The MBC is the lowest concentration of EO which 99.9% of the microorganisms were killed. The tests were carried out in triplicate.

### 2.7.3 Antifungal assay

Antifungal assay was performed by the agar medium assay (Yehouenou *et al.*, 2010). Yeast Extract Sucrose (YES) medium with different concentrations of essential oil (0.015, 0.025, 0.050, 0.075, 0.100, 0.150, 0.200, 0.250 $\mu L.mL^{-1}$) were prepared by adding appropriate quantity of EO and Tween 20 to melted medium, followed by manual rotation of Erlenmeyer to disperse the oil in the medium. About 20 mL of the medium were poured into glass Petri dishes (9 cm). Fungal isolates from orange juice on malt extract agar (MEA) are transplanted (subcultured), using a disc of 6 mm in diameter which carries spores from the anamorph mold, on the surface of a Petri dish containing the former medium YES and EO at different concentrations. Positive

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Control plates (without EO and inoculated following the same procedure) and negative control plates were also used. Plates were incubated at 25 °C for 5 days.

#### 2.7.4 Determination of the fongiostatic or fungicidal activity

With the experimental concentrations where neither growth nor germination was observed, the fungiostatic or fungicidal activity was tested. This assay consisted by taking the mycelial disc not germinated at the end of the incubation of the Petri dish and reintroducing it in a new culture medium (former one) without EO. If the mycelial growth is always inhibited, the plant extract is fungicidal at this concentration and allows the determination of the minimum fungicidal concentration (MFC). In the contrary case, it became fungiostatic activity which is related to the minimum inhibitory concentration (MIC) (Tomohiro, 1990).

### *2.8 Conservation of orange juice with essential oil*

To evaluate the conservation potentiality of the EO of *Cymbopogon citratus* L., juices extracted from oranges collected at Allada (South of Benin), Klouekanme (South of Benin) and Zakpota (Center of Benin) were mixed together to give a composite sample which was used for the tests. Five EO concentrations were tested. These are 0.025, 0.050, 0.062, 0.125 and 0.250 $\mu L.mL^{-1}$. These concentrations were chosen taking into account the high fragrant nature of the EO and different results about its antimicrobial properties, reported in the literature (Esteve and Frigola, 2008). A negative control (orange juice without EO) was also produced. Samples were placed at 25 °C. After 15 days of conservation at this temperature, microbiological and physicochemical qualities of conserved juice were then evaluated. The pH of the samples were determined in 10ml of orange juice using a digital pH-meter. Vitamin C (l-ascorbic acid) concentration was determined using method described by Adjou *et al.* (2013).

### *2.9 Statistical analysis*

Experiments were performed in triplicate, and data analyzed are means ± SE subjected to one-way Anova. Means are separated by the Tukey's multiple range test when Anova was significant ($P<0.05$) (SPSS 10.0; Chicago, IL, USA).

## 3 Results

The result of microbial analysis and isolation of fungi in pure culture revealed that orange juices collected from the main markets of south and center of Benin, were contaminated by microorganisms such as bacteria, yeast and fungi (Table 1). Bacteria isolates include *E.coli*, *Enterobacter aerogenes* , *Enterobacter cloacae* and *Staphylococcus aureus.*

High contamination rates of yeast and molds were obtained with the level ranging between $1x10^5$ and $3x10^5$ cfu / mL for yeasts and $1x10^2$ and $1.5x10^2$ cfu / mL for the mold, with the predominant presence of *Penicillium spp*, and *Cladosporium spp*. The results of the evaluation of the physicochemical characteristics of the fresh juice (Table 2) indicated that the

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pH of the juice were between 6.2 ± 0.3 and 6.8 ± 0.1, with a vitamin C content ranged from 26.03 ± 0.08 mg / 100 mL to 31.03 ± 0.05 mg / 100mL.

**Table 1:** Microbiological quality of investigated orange juice (cfu/mL).

| Microbiological parameters | Localities investigated | | |
|---|---|---|---|
| | Allada | Klouekanme | Zakpota |
| Total bacterial count | $3.83 \times 10^{5\,a}$ | $2.57 \times 10^{5\,b}$ | $2.99 \times 10^{5\,b}$ |
| Total coliforms count | $1 \times 10^{3\,a}$ | $1 \times 10^{3\,a}$ | $1 \times 10^{3\,a}$ |
| E.coli | $1^{a}$ | $0^{a}$ | $1^{b}$ |
| Enterobacter aerogenes | $0^{a}$ | $1^{b}$ | $1^{b}$ |
| Enterobacter cloacae | $1^{a}$ | $1^{a}$ | $1^{a}$ |
| Staphylococcus aureus | $1.87 \times 10^{5\,a}$ | $1.15 \times 10^{5\,a}$ | $1.6 \times 10^{5\,a}$ |
| Yeast count | $2 \times 10^{5\,a}$ | $3 \times 10^{5\,a}$ | $1 \times 10^{5\,a}$ |
| Fungi count | $1 \times 10^{2\,a}$ | $1 \times 10^{2\,a}$ | $1.5 \times 10^{2\,a}$ |

Values are means. The means followed by same letter in the same line are not significantly different according to ANOVA and Tukey's multiple comparison tests.

**Table 2:** Physicochemical quality of investigated orange juice

| Physicochemical parameters | Localities investigated | | |
|---|---|---|---|
| | Allada | Klouekanme | Zakpota |
| pH | $6.8 \pm 0.1^{a}$ | $6.2 \pm 0.3^{b}$ | $6.3 \pm 0.1^{b}$ |
| Vitamin C (mg/100ml) | $31.03 \pm 0.05^{a}$ | $26.03 \pm 0.08^{b}$ | $29.03 \pm 0.04^{b}$ |

Values are means. The means followed by same letter in the same line are not significantly different according to ANOVA and Tukey's multiple comparison tests.

By hydrodistillation, fresh leaves of *C. citratus* yielded 1.31% of EO. Chemical analysis by GC and GC-MS analysis of EO enabled the identification of 26 components, (Table 3) representing 98.1 % of the EO. In the volatile extract, different groups of terpenes and terpenoids were detected. The EO has chemical composition characterized by Myrcene (10.4 %), Neral (33.0%) and Geranial (41.3%) as major components.

The EO exhibited pronounced antibacterial activity against the growth of *Eschericha coli, Enterobacter aerogenes*, *Enterobacter cloacae* and *Staphylococcus aureus*. MIC value was 0.05 $\mu L.mL^{-1}$ for all bacteria tested. However, MBC values were 0.1 $\mu L \times mL^{-1}$ for *Eschericha coli, Enterobacter aerogenes*, *Enterobacter cloacae* and 0.15 $\mu L \times mL^{-1}$ for *Staphylococcus aureus.* The EO exhibited pronounced antifungal activity against the growth of *Penicillium spp* and



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*Cladosporium spp*. The MIC of the EO was found to be 0.15 µL x $mL^{-1}$ for *Penicillium spp* and 0.20 µL x $mL^{-1}$ for *Cladosporium spp*. The MFC was recorded to be µL x $mL^{-1}$ for *Penicillium spp* and *Cladosporium spp.*

**Table 3:** Chemical composition of investigated EO of *Cymbopogon citratus*

| Compound | Kovats Index (KI) | Percentage (%) |
|---|---|---|
| 6-méthyl-hep-5-en-2-one | 985 | 1.2 |
| Myrcene | 991 | 10.4 |
| Limonene | 1031 | - |
| (Z)-β-ocimene | 1036 | 0.2 |
| (E)-β-ocimene | 1047 | 0.2 |
| 6,7-èpoxymyrcene | 1091 | 0.2 |
| Pirillene | 1098 | 0.1 |
| Linallol | 1100 | 0.5 |
| 2,2-octa-3,4-dienal | 1106 | 0.1 |
| Cis-vervenol | 1140 | 0.1 |
| Trans-verbenol | 1144 | - |
| Menth-3-en-9-ol | 1150 | 0.1 |
| Citronella | 1153 | 0.4 |
| Cis-chrysanthenol | 1162 | 0.5 |
| Epoxy rose furane | 1170 | 0.2 |
| Nerol | 1231 | 0.3 |
| Neral | 1245 | 33.0 |
| Geraniol | 1256 | 6.6 |
| Geranial | 1276 | 41.3 |
| Formate of neryle | 1285 | 0.1 |
| Acetate of geranyle | 1378 | 2.4 |
| β-caryophyllene | 1419 | - |
| Oxyde of caryophyllene | 1587 | 0.1 |
| **Total** | | 98,1 |

Results obtained during storage tests of fresh juice with the EO of *C. citratus* at different concentrations (Table 4) indicated a strong antimicrobial activity of the EO against the spoilage flora of fresh orange juice. Indeed, with the essential oil concentration of 0.125 µL x $mL^{-1}$, there



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was a good antibacterial activity, mainly against *E. coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Staphylococcus aureus*.

**Table 4:** Microbiological quality of investigated orange juice after 15 days of conservation (UFC/ml).

| Parameters | Concentrations of essential oil (µl/ml) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.025 | 0.050 | 0.062 | 0.125 | 0.250 |
| Total bacterial count | $2 \times 10^{7\,a}$ | $3 \times 10^{5\,b}$ | $102^{c}$ | $10^{d}$ | $10^{d}$ | $07^{d}$ |
| Total coliforms count | $1 \times 10^{5\,a}$ | $10^{2\,b}$ | $10^{c}$ | $0^{d}$ | $0^{d}$ | $0^{d}$ |
| *E.coli* | $1 \times 10^{3\,a}$ | $10^{b}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ |
| *Enterobacter aerogenes* | $5 \times 10^{2\,a}$ | $10^{b}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ |
| *Enterobacter cloacae* | $2 \times 10^{2\,a}$ | $10^{b}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ |
| *Staphylococcus aureus* | $1.6 \times 10^{6}$ | $10^{2}$ | 0 | 0 | 0 | $0^{c}$ |
| Yeast count | $10^{5\,a}$ | $10^{3\,b}$ | $10^{c}$ | $10^{c}$ | $10^{c}$ | $0^{c}$ |
| Fungi count | $10^{2\,a}$ | $10^{2\,a}$ | $1.2 \times 10^{1\,b}$ | $10^{c}$ | $07^{c}$ | $0^{d}$ |

Values are means. The means followed by same letter in the same line are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table 5 presented the results of the evaluation of the physicochemical characteristics of the orange juice stored by adding EO after 15 days. These results indicated that, in orange juice stored with EO at the concentrations of 0.025 - 0.062 µL.mL$^{-1}$, there was a significant difference in pH and vitamin C content, after 15 days of storage. However, at the concentration of 0.250 µL x mL$^{-1}$, there was no significant difference in pH and vitamin C levels after 15 days of storage with a best vitamin C content of 28.06 ± 0.03 mg / 100mL.

**Table 5:** Physicochemical quality of investigated orange juice after 15 days of conservation

| Physicochemical parameters | Juice 's characteristics at the beginning of the conservation tests | Characteristics of the juices after 15 days of conservation | | | | | |
|---|---|---|---|---|---|---|---|
| | | Concentrations of essential oil (µl/ml) | | | | | |
| | | 0 | 0.025 | 0.050 | 0.062 | 0.125 | 0.250 |
| pH | $6.2\pm0.3^{a}$ | $3.4\pm0.1^{b}$ | $3.9\pm0.4^{b}$ | $4.4\pm0.6^{b}$ | $4.9\pm0.2^{c}$ | $5.3\pm0.6^{c}$ | $6.4\pm0.1^{d}$ |
| Vitamin C (%) | $29.02\pm0.01^{a}$ | $1.06\pm0.03^{b}$ | $1.07\pm0.09^{b}$ | $8.01\pm0.04^{c}$ | $12.08\pm0.07^{c}$ | $21.06\pm0.08^{d}$ | $28.06\pm0.03^{e}$ |

Values are means. The means followed by same letter in the same line are not significantly different according to ANOVA and Tukey's multiple comparison tests



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## 4 Discussion

The beneficial activities of orange juice components for human health are primarily attributed to their antioxidant capacity (Somogy, 1996). The results obtained on the evaluation of the physicochemical quality of fresh orange juice, indicated that they are a good source of vitamin C, natural and easily accessible by the public. Indeed, vitamin C is an important vitamin and plays an important role in human health and preservation. It is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported (Adjou *et al.*, 2012). The consumption of foods rich in antioxidant substance may contribute to the preservation of cell's oxidation (FAO, 1998). In the food industry, vitamin C is used as a food additive (Leclerc *et al.*, 2002). It is frequently added to fruit juices to preserve and protect them from any color changes.

However, the pH of these fresh juices, being closely to neutrality, makes them subject to a potential risk of contamination by microorganisms. Indeed, the results of microbiological analyzes on the quality of fresh orange juice indicated a contamination by bacteria from gastroenteritis tropism, such as, *E. coli, Enterobacter aerogenes, Enterobacter cloacae* and bacteria from mucocutaneous tropism, such as *Staphylococcus aureus*. This high total bacterial and coliform count may have been as a result of the low level of hygiene maintained during the sale of oranges. Indeed, the bad oranges handling conditions, induced the onset of injury to the pericarp, and the exposure of orange stock on ground for sale, promote contamination and penetration of microorganisms in oranges.

The detection of *Escherichia coli*, which is enteric bacteria, confirmed the poor hygienic practice during the sale of oranges. The isolation of coliforms from orange juices, pose a serious threat to food safety, due to the fact that fruit juice are ready to eat foods, which are consumed without further processing. Similar results were found on the other street foods and the germs most identified in these foods were mainly *Staphylococci* and enterobacteria (Dahouenon-Ahoussi *et al.*, 2012). According to Food and Agriculture Organization (FAO)/ World Health Organization (Esteve and Frigola, 2008), epidemiological data in hospital showed a prevalence of 19% of diarrheal disease worldwide and bacterial diarrhea was estimated between 20 and 70% of the cases. The causes were related to poor hygiene found in the assessment of hazards and identification of critical points in the food processing chain (Somogyi, 1996). However, fungal contamination of samples is higher, and can constitute a health risks to the consumer because of toxigenicity of molds, but also constitutes an important factor of impaired marketability of the product. These factors (yeasts and molds) are increasingly taken into account nowadays, when developing antimicrobial products to maintain quality of highly perishable foodstuffs

EO are natural mixtures of hydrocarbons and oxygen (alcohols, aldehydes, ketones, carboxylic acids, esters, and lactones) containing organic substances of plants. Their constituents and derivatives have a long history of application as antimicrobial agents in the areas of food preservation and medicinal antimicrobial production (Voda *et al.*, 2003). The present study also explores the bioefficacy of EO of *C. citratus* as the promising plant-based antimicrobial against oranges juice-infecting fungi and bacteria. This EO was found to be effective against bacteria, and fungi infecting orange juice. This bioefficacy may be due to the presence of some highly fungitoxic components in the oil such as terpenoids. Indeed, terpenoids are a large group of antimicrobial compounds that are active against a broad

spectrum of microorganisms (Dorman, 2000). Their antimicrobial activities are linked to their functional groups and it has also been reported that the hydroxyl group of phenolic terpenoids and the presence of delocalized electrons are important for the antimicrobial activity (Suhr *et al.*, 2003). Then, biological activities of EO depends on the qualitative and quantitative characteristics of their components (Matasyoh, 2011). In our study, GC–MS data, depicted remarkable variation with the earlier reports on the oil (Prakash *et al.*, 2010). The chemical profile of essential oils, is also reported to be influenced by the harvest period, geographical, climatic conditions, and the amount of active constituents (Soumanou and Adjou, 2016). Thus, the biologically active EO should be qualitatively standardized before their recommendation for practical exploitation as has been done in the present investigation. The results of conservation of orange juice with EO confirmed the bioefficacy potential of this EO and opened new perspectives in the use of natural plant extracts as an opportunity to avoid synthetic chemical preservatives, and offers novel approach to the management of storage fungi. It was a promising method for preserving stored products in rural areas, which do not have access to modern storage system.

## 5 Conclusions

This work underlined the bioactivity of essential oil of fresh leaves of *C. citratus* from Benin as a promising plant-based antimicrobial against orange juice-infecting fungi and bacteria. Different major components such as Myrcene (10.4%), Neral (33.0%) and Geranial (41.3%) were present in the volatile extract. Based on its antibacterial and antifungal activities, this natural plant product may successfully replace synthetic chemicals, and provide an alternative method in the stabilization of fresh orange juice, as well as other agricultural commodities of nutritional significance from microbiological spoilage alteration.

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## References

Adams R.P., 2007. Identification of essential oil components by gas chromatography / mass spectrometry. Allured, Carol Stream.

Adjou E. S., Amamion H., Tchobo F. P., Aissi V. M., Soumanou M.M., 2013. Extraction assistée par enzyme du jus de la pulpe fraîche du rônier (Borassus aethiopum Mart.) acclimaté au Benin : caractérisation physico-chimique et microbiologique. Int. J. Biol. Chem. Sci. 7(3), 1135-1146.

  ISSN: 2283-3927

Adjou E.S., Yehouenou B., Sossou C.M., Soumanou M.M., de Souza C.A., 2012. Occurrence of mycotoxins and associated mycoflora in peanut cakes products (kluiklui) marketed in Benin. Afr J Biotechnol 11(78), 14354–14360.

Bajpai K.V., Dung N.T., Kwon J.O., Kang S.C., 2008. Analysis and the potential application of essential oil and leaf extracts of Silene Americana L. to control food spoilage and food-borne pathogens. J. Food Technol., 227: 1613-1620.

Bankole S., Mabekoje O.O., 2004. Mycoflora and occurrence of aflatoxin B1 in dried yam chips from markets in Ogun and Oyo States, Nigeria. Mycopathologia, 157, 111-115.

Barminas J.T., Charles M., Emmanuel D., 1998. Mineral Composition of Non- conventional Leafy Vegetables. Plant Foods for Human Nutrition 53 (1), 29-36.

Bazemore R., Goodner K., Rouseff R., 1999. Volatiles from Unpasteurized and Excessively Heated Orange Juice Analyzed with Solid Phase Microextraction and GC-Olfactometry. J. Food. Sci. 64 (5), 800-803.

Dahouenon-Ahoussi E., Degnon R. G., Adjou E. S., Sohounhloue D.C.K., 2012. Stabilisation de la bière produite à partir de matières amylacées locales (Sorghum bicolor et Musa acuminata) par adjonction de l'huile essentielle de Cymbopogon citratus. Journal of Applied Biosciences 51, 3596 – 3607.

de Billerbeck V.G., Roques C.G., Bessière J.M., Fonvieille J.L, Dargent R., 2001. Effect of Cymbopogon nardus (L) W. Watson. essential oil on the growth and morphogenesis of Aspergillus niger. Can J Microbiol 47, 9–17.

Dorman H.J.D., Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils, J. Appl.Microbiol., 88, 308–316. 2000.

Esteve J.M., Frigola A., 2008.The effects of thermal and Non-thermal Processing on Vitamin C, Carotenoids, Phenolic Compounds and Total Antioxydant Capacity in Orange juice. Tree and forestry Science and Biotechnology, 2(1), 128-134.

FAO/WHO, 1998. Forty-ninth meeting of the Joint Expert Committee on Food Additives. Food Agric. Organ. United Nation. Rome, p. 140.

Hammer K.A., Carson C.F., Ridley C.V., 1999. Antimicrobial activity of essential oils and other plants extract. Journal of Applied Microbiology, 86, 985-990.

Hidalgo E., Moor, D., Patourel G., 1998.The effect of different formulations of Beauveria bassiana on Sitophilus zeamais. Journal of Stored Products Research, 34, 171–179.

Leclerc H., Schwarrtzbrod L., Dei-Cas E., 2002. Microbial agents associated with waterborne diseases. Crit. Rev. Microbiol., 28,371-409.

Matasyoh J.C., Wagara I.N., Nakavuma J.L., Kiburai A.M., 2011. Chemical composition of Cymbopogon citratus essential oil and its effect on mycotoxigenic Aspergillus species. African Journal of Food Science, 5(3), 138-142

Melo S.F., Soares S.F., Costa D.R., Silva D.C., Oliveira D.M., Bezerra R.J., 2001. Effect of the *Cymbopogon citratus, Maytenus ilicifolia* and *Baccharis genistelloides* extracts against the stannous chloride oxidative damage in *Escherichia coli*. Mutat Res., 496, 33–41.

  ISSN: 2283-3927

Misrha A.K., Dubey N.K., 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. Appl Environ Microbiol., l60, 1101–1106.

Moosavy M.H., Basti A.A., Ali M., 2008. Effect of Zataria multiflora Boiss. Essential oil and nisin on Salmonella typhimurium and Staphylococcus aureus in a food model system and on the bacterial. International Journal of Food Microbiology, 43, 69–76.

Oussou K.R., Kanko C., Guessend N., Yolou, S., Koukoua G., Dosso M., N'guessan Y.T., Figueredo G., Chalchat J-C., 2004. Activités antibactériennes des huiles essentielles de trois plantes de Côte d'Ivoire. C.R. Chim., 7, 1081-1086.

Prakash B., Shukla R., Singh P., Mishra PK., Dubey N.K., Kharwar R.N., 2010. Efficacy of chemically characterized Ocimum gratissimum L. essential oil as an antioxidant and a safe plant based antimicrobial against fungal and aflatoxin B1 contamination of spices, Food Res. Int., 10, 128 -132.

Singh K., Frisvad J.C., Thrane U., Mathu S.B., 1991. An illustrated manual on identification of some seed borne Aspergilli, Fusaria, Penicillia and their mycotoxins. Heller up, Denmark: Danish Government, Instituteof seed pathology for developing countries. p 133.

Somogyi L.P., 1996. Direct food additives in food processing. In L. P. Somogyi, D. M. Barrett, & Y. H. Hui (Eds.), Processing Fruits: Science and Technology, Biology, Principles, and Application. Western Hemisphere 7 Technomic Publishing, 1, 342-345.

Soumanou M.M. and Adjou E.S., 2016. Sweet Fennel (Ocimum gratissimum) Oils. In:Preedy, V.R. (Ed.), Essential Oils in Food Preservation, Flavor and Safety. Academic Press, 765–773.

Suhr K.I., Nielsen P.V., 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi, J. Appl. Microbiol., 94(4), 665–674.

Tomohiro S., 1990. Laboratory Manual for Food analysis", Technical Cooperation Project of Jomo Kenyatta 76p.

Tran M. T. T., Farid M., 2004. Ultraviolet treatment of orange juice. Innovative Food Science and Emerging Technologies 5, 495– 502.

Voda K., Boh B., Vrta-cnik M., Pohleven F., 2003. Effect of the antifungal activity of oxygenated aromatic essential oil compounds on the white-rot Trametes versicolor and the brown-rot Coniophora puteana, Int. Biodeterior. Biodegrad., 51, 51–59.

Yèhouenou B., Noudogbèssi J.P., Sessou P., Avlessi F., Sohounhloué D., 2010. Etude chimique et activités antimicrobiennes d'extraits volatils des feuilles et fruits de Xylopia aethiopica (DUNAL) A. Rich. contre les pathogènes des denrées alimentaires. J. Soc Ouest – Afr. Chim., 029, 19-27.

  ISSN: 2283-3927